LAP-6, the mutant version with the catalytic cysteine residue inactivated (ICE LAP-6 mt) or ICE as described elsewhere herein. Percent apoptotic cells represents the mean value from three independent experiments. As predicted, overexpression of the mutant form of ICE LAP-6 did not induce apoptotic changes in MCF7 cells (Figure 6). Furthermore, these results demonstrate that an ICE/Ced-3 family member containing an active site QACGG (SEQ ID NO:11) pentapeptide (rather than QACRG (SEQ ID NO:10)) may still possess apoptosis-inducing potential and presumably enzymatic activity.

Please replace the paragraph beginning at page 61, line 12, with the following rewritten paragraph:

Provided by the present invention is a novel member of the ICE/Ced-3 family of cysteine proteases. ICE LAP-6 has a unique active site QACGG (SEQ ID NO:10) pentapeptide and is classified in the subfamily most related to Ced-3 and Yama. Ectopic expression of ICE LAP-6 in mammalian cells causes apoptosis.

REMARKS

The Substitute Specification has been reformatted to correct the margins, as requested in the Notice to File Corrected Application Papers. The amendments to the specification correct the priority claim, correct the reference to the Figures and their subparts, and add more complete reference to the sequence listing. The paragraph at page 8, lines 7-12, has been canceled because the described Figure 2 does not exist. Previously labeled Figures 3-7 have been relabeled as Figures 2-6 throughout the specification. No new matter has been added by way of any of the amendments.

Although the Notice to File Corrected Application Papers stated that Applicants had failed to submit a computer readable form (CRF) of the sequence listing so as to comply with 37 C.F.R. 1.821(e), Applicants had in fact filed on September 24, 2001, together with the instant application, a Statement Under 37 C.F.R. 1.821(e) requesting the use of the CRF from a related application. Nonetheless, in the interest of furthering prosecution, a Substitute Sequence Listing and corresponding CRF are submitted herewith. The sequence information in the Substitute Sequence Listing and CRF has not been altered from that most recently filed in priority applications Ser. No. 09/300,328 or Ser. No. 08/852,936.

Applicants believe that all the requirements of the Notice to File Corrected Application Papers now have been satisfied and respectfully request that the instant application be examined on the merits.

CONCLUSION

Applicants respectfully request that the above-made remarks be entered and made of record in the file history of the instant application. If there are any fees due in connection with the filing of this paper, please charge the fees to Deposit Account No. 08-3425. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to Deposit Account No. 08-3425.

Respectfully submitted,

Date: 11 March 2003

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VIA HAND DELIVERY March 11, 2003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Dixit et al.

Application Number: 09/961,201

Filed: September 24, 2001

Group Art Unit: To be assigned

Examiner: To be assigned

Atty. Docket No.: PF335D2

Title: *Interleukin-1 β Converting Enzyme Like Apoptotic Protease-6*

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification

The paragraph beginning at page 1, line 5, is amended as follows:

This application is a divisional of U.S. Application Ser. No. 09/300,328, filed April 27, 1999, now U.S. Patent No. 6,294,169, which is a divisional of U.S. Application Ser. No. 08/852,936, filed May 8, 1997, now U.S. Patent No. 6,010,878, which claims the benefit of U.S. Provisional Application Nos. 60/018,961 filed June 5, 1996, 60/020,344 filed May 23, 1996 and 60/017,949 filed May 20, 1996.

The paragraph beginning at page 4, line 24, is amended as follows:

Toward these ends, and others, it is an object of the present invention to provide polypeptides, inter alia, that have been identified as novel ICE LAP-6 by homology between the amino acid sequence set out in Figure 1 or the polypeptide encoded by the deposited clone and known amino acid sequences of other proteins such as those sequences set out in ~~Figure 2~~ Figures 2A-2C.

The two paragraphs beginning at page 5, line 1, are amended as follows:

In a particular preferred embodiment of this aspect of the invention the polynucleotide comprises the region encoding human ICE LAP-6 set forth in ~~Figure 3~~ Figures 2A-2C.

In accordance with this aspect of the present invention there is provided an isolated nucleic acid molecule encoding a mature polypeptide expressed by the human cDNA in ~~Figure 3~~ Figures 2A-2C or derived using the primers set forth in Example 1, or

a polynucleotide encoding the polypeptide in Figure 1 or derived from the polypeptide encoded by the deposited clone.

The seven paragraphs beginning at page 8, line 4, are amended as follows:

Figure 1 [SEQUENCE ID NO. 1] shows the predicted amino acid sequence of human ICE LAP-6. The active site pentapeptide QACGG (SEQ ID NO:11) is underlined. Putative amino acid (Asp) cleavage sites are indicated with bold letters.

[Delete paragraph at page 8, lines 7-12.]

~~Figure 3~~ Figures 2A-2C [SEQUENCE ID NO. 2] ~~shows~~ show a nucleic acid sequence of human ICE LAP-6.

Figure ~~4~~ 3 [SEQUENCE ID NO. 3] shows a nucleic acid sequence variant derived from human ICE LAP-6.

Figure ~~5~~ 4 [SEQUENCE ID NO. 4] shows an amino acid sequence variant derived from human ICE LAP-6.

Figure ~~6~~ 5 shows phylogenetic analysis of the ICE/ced-3 gene family.

Figure ~~7~~ 6 shows MCF7 the results of an analysis of breast carcinoma cells transiently transfected demonstrating that over-expression of ICE LAP-6 induces cell death in mammalian cells.

The paragraph beginning at page 17, line 2, is amended as follows:

The present invention relates to novel ICE LAP-6 polypeptides and polynucleotides, among other things, as described in greater detail below. In particular, the invention relates to polypeptides and polynucleotides of a novel human ICE LAP-6, which is related by amino acid sequence homology to human interleukin-1 beta converting enzyme apoptosis protease polypeptides. The invention relates especially to ICE LAP-6 having the nucleotide and amino acid sequences set out in Figures 1 and ~~3~~ 2A-2C respectively. It will be appreciated that the nucleotide and amino acid sequences set out in Figures ~~3~~ 2A-2C and 1 respectively, were obtained by sequencing the cDNA obtained from a human K562 (erythroleukemia) cell line cDNA library.

The paragraph beginning at page 17, line 28, is amended as follows:

Human ICE LAP-6 of the invention is structurally related to other proteins of the human interleukin-1 beta converting enzyme apoptosis protease family, as shown by the

results of sequencing the cDNA encoding human ICE LAP-6 in Figure 1. The cDNA of ~~Figure 3~~ Figures 2A-2C was obtained as described in Example 1. The polypeptide of Figure 1 and the polypeptide encoded by the deposited clone each are proteins which have a deduced molecular weight of about 45.8 kDa.

The paragraph beginning at page 18, line 9, is amended as follows:

The coding sequence which encodes the polypeptide may be identical to the coding sequence of the polynucleotide derived using the primers set forth in Example 1, or the polynucleotide of ~~Figure 3~~ Figures 2A-2C. It also may be a polynucleotide with a different sequence, which, as a result of the redundancy (degeneracy) of the genetic code, encodes the polypeptide of the cDNA of Figure 1 or the polypeptide encoded by the deposited clone.

The paragraph beginning at page 20, line 28, is amended as follows:

Particularly preferred embodiments in this respect, moreover, are polynucleotides which encode polypeptides which retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNA of ~~Figure 3~~ Figures 2A-2C encoded by the polynucleotide sequence of the deposited clone, or derived using the primers set forth in Example 1.

The paragraph beginning at page 25, line 19, is amended as follows:

Also among preferred embodiments of this aspect of the present invention are polypeptides comprising fragments of ICE LAP-6, most particularly fragments of the ICE LAP-6 having the amino acid set out in Figure 1 or the amino acid sequence of the polypeptide encoded by the deposited clone, and fragments of variants and derivatives of the ICE LAP-6 of Figure 1 or the polypeptide encoded by the deposited clone, such as, for example the amino acid sequence of Figure ~~5~~ 4 .

The paragraph beginning at page 27, line 25, is amended as follows:

Further preferred regions are those that mediate activities of ICE LAP-6. Most highly preferred in this regard are fragments that have a chemical, biological or other activity of ICE LAP-6, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Highly preferred in this regard are fragments that

contain regions that are homologs in sequence, or in position, or in both sequence and to active regions of related polypeptides, such as the related polypeptides set out in ~~Figure 2~~ Figures 2A-2C, which include human interleukin-1 beta converting enzyme apoptosis proteases. Among particularly preferred fragments in these regards are truncation mutants, as discussed above.

The paragraph beginning at page 28, line 3, is amended as follows:

It will be appreciated that the invention also relates to, among others, polynucleotides encoding the aforementioned fragments, polynucleotides that hybridize to polynucleotides encoding the fragments, particularly those that hybridize under stringent conditions, and polynucleotides, such as PCR primers, for amplifying polynucleotides that encode the fragments. In these regards, preferred polynucleotides are those that correspondent to the preferred fragments, as discussed above. Preferred polynucleotides fragments may be derived from the sequences of ~~Figure 3 and 4~~ Figures 2A-2C.

The paragraph beginning at page 54, line 28, is amended as follows:

Members of the ICE/ced-3 gene family are belived to be effector components of the cell death machinery. Herein this Example, a novel member of this family designated ICE LAP-6 is characterized. By phylogenetic analysis, ICE LAP-6 is classified into the Ced-3 subfamily which includes Ced-3, Yama/CPP32/apopain, Mch2 and ICE LAP-3/Mch3/CMH-1. ICE LAP-6 contains an active site QACGG (SEQ ID NO:11) pentapeptide, rather than the QACRG (SEQ ID NO:10) pentapeptide shared by other family members. Overexpression of ICE LAP-6 induces apoptosis in MCF7 breast carcinoma cells. ICE LAP-6 is also proteolytically processed into an active cysteine protease by granzyme B, an important component of cytotoxic T cell-mediated apoptosis. Once activated, ICE LAP-6 is able to cleave the death substrate poly (ADP-ribose) polymerase (PARP) into signature apoptotic fragments.

The paragraph beginning at page 58, line 11, is amended as follows:

A blast search of GenBank protein data base revealed that the predicted protein sequence of ICE LAP-6 has significant similarity to the members of the ICE/Ced-3 family, particularly in the regions corresponding to the active subunits of ICE (Thomberry, N. A.,et al (1992) Nature 356, 768-774). In this region, ICE LAP-6 shares

31% sequence identity (55% sequence similarity) with the C. elegans CED-3 protein, 33% identity (52% sequence similarity) with ICE-LAP3, 30% identity (56% similarity) with Mch2a and 29% sequence identity (52% similarity) with Yama. ICE LAP-6 also has 25%-28% sequence identity with ICE and the ICE-related genes, ICE rel II and ICE rel III. Phylogenetic analysis of the ICE/ced-3 gene family showed that ICE LAP-6 is a member of the Ced-3 subfamily which includes Yama, ICE-LAP3, and Mch2 (Figure ~~6~~ 5). Like Ced-3, ICE LAP-6 contains a long N-terminal putative prodomain. Based on the x-ray crystal structure of ICE (Walker, N. P. C. et al, (1994) Cell 78, 343-352; Wilson, K. P., et al (1994) Nature 370, 270-275), the amino acid residues His237, Gly238, Cys285 of ICE are involved in catalysis, while the residues Arg179, Gln283 and Arg341 form a binding pocket for the carboxylate side chain of the P1 aspartic acid. These six residues are conserved in all ICE/Ced-3 family members thus far cloned as well as in ICE LAP-6. However, residues that form the P2-P4 binding pockets are not widely conserved among family members, suggesting that they may determine substrate specificity. Surprisingly, ICE LAP-6 contains a unique active site pentapeptide QACGG (SEQ ID NO:11), instead of the QACRG (SEQ ID NO:10) shared by other family members (~~Figure 2~~ Figures 2A-2C).

The paragraph beginning at page 59, line 10, is amended as follows:

To study the functional role of ICE LAP-6, MCF7 breast carcinoma cells were transiently transfected with an expression vector encoding the full-length ICE LAP-6 protein (ICE LAP-6-flag) and subsequently assessed for apoptotic features. Like the other ICE/Ced-3 family members, expression of ICE LAP-6 caused cell death (Figure ~~7~~ 6) The ICE LAP-6-transfected MCF7 cells displayed morphological alterations typical of adherent cells undergoing apoptosis, becoming rounded, condensed, and detaching from the dish. ICE LAP-6 induced apoptosis was inhibited by the broad spectrum ICE inhibitor z-VAD fmk (Pronk, G. J., (1996) Science 271, 808-810). To determine whether the amino acid residue Cys286, corresponding to the catalytic Cys285 of ICE, was essential for apoptotic activity, a mutant form of ICE LAP-6 was generated in which the cysteine residue was altered to an alanine. MCF7 breast carcinoma cells were transiently transfected with the reporter gene b-galactosidase and either C-terminal flag-tagged ICE LAP-6, the mutant version with the catalytic cysteine residue inactivated (ICE LAP-6 mt) or ICE as described elsewhere herein. Percent apoptotic cells represents the mean value

from three independent experiments. As predicted, overexpression of the mutant form of ICE LAP-6 did not induce apoptotic changes in MCF7 cells (Figure ~~7~~ 6). Furthermore, these results demonstrate that an ICE/Ced-3 family member containing an active site QACGG (SEQ ID NO:11) pentapeptide (rather than QACRG (SEQ ID NO:10)) may still possess apoptosis-inducing potential and presumably enzymatic activity.

The paragraph beginning at page 61, line 12, is amended as follows:

Provided by the present invention is a novel member of the ICE/Ced-3 family of cysteine proteases. ICE LAP-6 has a unique active site QACGG (SEQ ID NO:10) pentapeptide and is classified in the subfamily most related to Ced-3 and Yama. Ectopic expression of ICE LAP-6 in mammalian cells causes apoptosis.